8-2003

How Rapidly Can Maternal Behavior Affecting Primary Sex Ratio Evolve in a Reptile with Environmental Sex Determination?

Carrie L. Morjan
Iowa State University

How Rapidly Can Maternal Behavior Affecting Primary Sex Ratio Evolve in a Reptile with Environmental Sex Determination?

Abstract
Theoretical models identify maternal behavior as critical for the maintenance and evolution of sex ratios in organisms with environmental sex determination (ESD). Maternal choice of nest site is generally thought to respond more rapidly to sex ratio selection than environmental sensitivity of offspring sex (threshold temperatures) in reptiles with temperature-dependent sex determination (TSD, a form of ESD). However, knowledge of the evolutionary potential for either of these traits in a field setting is limited. I developed a simulation model using local climate data and observed levels of phenotypic variation for nest-site choice and threshold temperatures in painted turtles (*Chrysemys picta*) with TSD. Both nest-site choice and threshold temperatures, and hence sex ratios, evolved slowly to simulated climate change scenarios. In contrast to expectations from previous models, nest-site choice evolved more slowly than threshold temperatures because of large climatic effects on nest temperatures and indirect selection on maternally expressed traits. A variant of the model, assuming inheritance of nest-site choice through natal imprinting, demonstrated that natal imprinting inhibited adaptive responses in female nest-site choice to climate change. These results predict that females have relatively low potential to adaptively adjust sex ratios through nest-site choice.

Keywords
temperature-dependent sex determination, sex ratio evolution, maternal effects, climate change, turtles

Comments
This article is published as Morjan, Carrie L. "How rapidly can maternal behavior affecting primary sex ratio evolve in a reptile with environmental sex determination?." *The American Naturalist* 162, no. 2 (2003): 205-219. doi: 10.1086/376583. Posted with permission.

VOL. 162, NO. 2 THE AMERICAN NATURALIST AUGUST 2003

How Rapidly Can Maternal Behavior Affecting Primary Sex Ratio Evolve in a Reptile with Environmental Sex Determination?

Carrie L. Morjan*

Department of Zoology and Genetics, Iowa State University, Ames, Iowa 50011-3223

Submitted June 27, 2002; Accepted December 30, 2002; Electronically published July 21, 2003

ABSTRACT: Theoretical models identify maternal behavior as critical for the maintenance and evolution of sex ratios in organisms with environmental sex determination (ESD). Maternal choice of nest site is generally thought to respond more rapidly to sex ratio selection than environmental sensitivity of offspring sex (threshold temperatures) in reptiles with temperature-dependent sex determination (TSD, a form of ESD). However, knowledge of the evolutionary potential for either of these traits in a field setting is limited. I developed a simulation model using local climate data and observed levels of phenotypic variation for nest-site choice and threshold temperatures in painted turtles (*Chrysemys picta*) with TSD. Both nest-site choice and threshold temperatures, and hence sex ratios, evolved slowly to simulated climate change scenarios. In contrast to expectations from previous models, nest-site choice evolved more slowly than threshold temperatures because of large climatic effects on nest temperatures and indirect selection on maternally expressed traits. A variant of the model, assuming inheritance of nest-site choice through natal imprinting, demonstrated that natal imprinting inhibited adaptive responses in female nest-site choice to climate change. These results predict that females have relatively low potential to adaptively adjust sex ratios through nest-site choice.

Keywords: temperature-dependent sex determination, sex ratio evolution, maternal effects, climate change, turtles.

Maternal effects have important, although sometimes nonintuitive, evolutionary consequences (Kirkpatrick and Lande 1989; Wade 1998). Among these effects is maternal behavior affecting offspring phenotypes, which may play an important role in adaptation and evolutionary dynamics of populations. In reptiles with temperature-dependent sex determination (TSD), selection on two traits, maternal nesting behavior and thermal sensitivity of offspring sex, is important for maintaining sex ratios in changing thermal environments (Bulmer and Bull 1982; Bull 1983). Which of the two traits is more likely to respond to selection has long been debated (Bull et al. 1982*a*, 1982*b*; Janzen 1992; Rhen and Lang 1998; Janzen and Morjan 2001), but environmental variation mediating these traits has played a key role in these discussions (see, e.g., Bull et al. 1982*a*; Janzen 1992).

Temperature-dependent sex determination has persisted in ancient taxa that have undoubtedly endured substantial environmental upheaval (Janzen and Paukstis 1988, 1991*a*, 1991*b*). However, changes in egg incubation temperatures as small as 1°–2°C drastically skew offspring sex ratios in the laboratory (Bull 1980; Ewert and Nelson 1991) and in the field (Bull 1985; Janzen 1994*a*; Weisrock and Janzen 1999). The only long-term data correlating local climatic condition with annual cohort sex ratios in a reptile with TSD suggest that skewed primary sex ratios are likely to result from rapid and relatively small climatic changes ($\leq$2°C; Janzen 1994*a*). Such skewed sex ratios, should they persist to adulthood, are thought to be maladaptive; genes causing individuals to produce the rarer sex should increase in frequency through frequency-dependent selection (Fisher 1930).

Although substantial among-clutch variation in thermal sensitivity of offspring sex exists for several species exhibiting TSD, its significance for microevolution of sex ratio has been thought to be relatively low (Bull et al. 1982*a*; Janzen 1992; but see Rhen and Lang 1998). Eggs incubated at constant temperatures in the laboratory exhibit high heritabilities for sex ratio, but effective heritabilities for sex ratio in field-incubated nests are severely diminished as a result of environmental sources of sex ratio variation (0.03–0.06; Bull et al. 1982*a*; Janzen 1992). Maternal choice of thermal environments for nests has thus been

* Present address: Department of Biology, Indiana University, Jordan Hall 142, Bloomington, Indiana 47405; e-mail: cmorjan@bio.indiana.edu.

Am. Nat. 2003. Vol. 162, pp. 205–219.

thought to be more likely to respond to selection than embryonic response to temperature in organisms with TSD (Bull et al. 1982*a*; Bulmer and Bull 1982).

Nevertheless, few studies have addressed differences among individual females in nest-site selection to evaluate whether selection may act on such differences in the maintenance and evolution of sex ratios. In one study, females from a laboratory colony of leopard geckos (*Eublepharis macularius*) exhibiting TSD did not differ statistically in their choice of nest temperatures, although an upper limit of the heritability for nest temperature was estimated to be as high as 0.20 (Bull et al. 1988). In a second laboratory study, Bragg et al. (2000) detected significant differences in nest-site temperatures among individual female *E. macularius* and in a second species of gecko exhibiting TSD (*Hemitheconyx caudicinctus*). More recently, female painted turtles (*Chrysemys picta*) exhibited a repeatability of nest-site selection ($R = 0.18$–0.21) to microhabitats affecting nest temperatures and offspring sex ratios in a natural population with TSD (Janzen and Morjan 2001). Since repeatability estimates are thought to provide an upper estimate of the heritability for the trait (Boake 1989; Arnold 1994), this information suggests a promising role for maternal nesting behavior to manipulate offspring sex ratios.

Long-term data on a population of painted turtles *C. picta* provide the opportunity to model the relative evolutionary potential of nest-site choice and thermal sensitivity of offspring sex. In this study, I developed a simulation model based on population parameters of *C. picta* to evaluate the relative evolutionary potential for nest-site choice and thermal sensitivity of offspring sex, while including levels of thermal variation in field-incubated nests observed both within and among nesting seasons. Two variations of the model were constructed, one assuming an additive genetic basis for both nest-site choice and thermal sensitivity of offspring sex and the other assuming inheritance of nest-site choice through natal imprinting to nest microenvironments.

Methods

Study Organism and Focal Population

Chrysemys picta is a common North American freshwater turtle that inhabits a wide latitudinal range across varying climatic regimes (Ernst et al. 1994). Data were derived from a well-studied nesting population of painted turtles (*C. picta*) at the Thomson Causeway Recreation Area in Whiteside County, Illinois, from 1988 through 1999 (Janzen 1994*a*, 1994*b*; Weisrock and Janzen 1999; Janzen and Morjan 2001; Valenzuela and Janzen 2001). The nesting area is a campsite that is level, is fairly uniform in soil moisture levels and surface vegetation height (short grass), and has trees providing a wide range of thermal environments for nesting (Janzen 1994*b*). Annual offspring sex ratios are highly correlated with mean July air temperatures experienced at the site during the egg incubation period (Janzen 1994*a*; F. J. Janzen, unpublished data). Within years, offspring sex ratios in this population are also correlated with percentage of canopy vegetation cover over nests from the south and west directions (Janzen 1994*b*). However, this correlation exists only during years experiencing typical climatic conditions (Janzen 1994*b*; Valenzuela and Janzen 2001); extreme climatic conditions override the effects of vegetation cover on offspring sex ratios, producing unisexual or nearly unisexual yearly cohorts (Janzen 1994*a*).

Within years, nest temperatures are inversely correlated with the percentage of canopy vegetation cover over the nests, such that nests under high vegetation cover tend to be cooler (Weisrock and Janzen 1999; Morjan and Janzen 2003). Although variation in mean July air temperatures affects the intercept of the linear relationship between canopy vegetation and nest temperatures, the slopes remain parallel across years (Morjan and Janzen 2003). Nesting females exhibit repeatability in terms of vegetation cover over nests, which is not explained by nest-site fidelity on a geographic scale (Janzen and Morjan 2001; Valenzuela and Janzen 2001).

Details for collecting and incubating *Chrysemys picta bellii* eggs from the study site in Illinois during 1998 are described in a separate study evaluating among-population variation in sex determination (Morjan 2002). Offspring sex ratios as a function of clutch and temperature are displayed in table 1. The transitional range of threshold temperatures (TRT), which describes the range of incubation temperatures producing mixed sex ratios (Mrosovsky and Pieau 1991), was calculated using maximum likelihood (program 1.3 for Mac; Girondot 1999). Data available from the literature were also used to calculate TRT for two other populations of *C. picta* located in Wisconsin and Tennessee (Bull et al. 1982*b*). Higher values of TRT suggest wider ranges of temperatures where genetic variation in sex ratios can be expressed (Chevalier et al. 1999).

The Model

Model Development and Life Cycle. The yearly life cycle for the simulation model is depicted in figure 1. Modeling nest temperatures within and across years is discussed as follows. Mean July air temperatures taken from local data loggers and the percentage of vegetation cover from the south and west over the nest were significant sources of variation for 94 nest temperatures taken from 1995 to 1999

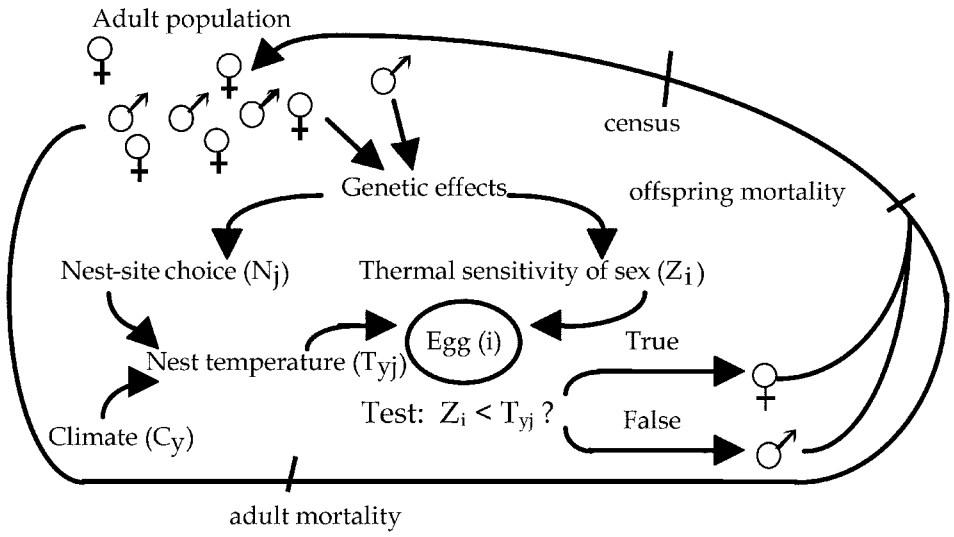

Figure 1: Simulated life cycle of *Chrysemys picta*

(multiple regression, $F = 122.77$, df $= 2, 91$, $r^2 = 0.73$, $P < .0001$). Therefore, these two variables were used to derive a model for nest temperature of nest j (T_{yj}) as a linear function of yearly climatic condition (C_y) for year y and microhabitat of the nest (N_j):

$$T_{yj} = \alpha + \beta C_y + \gamma N_j + \varepsilon, \tag{1}$$

where β and ε are the intercept and an error effect, respectively.

Threshold temperature (Z_i) was defined as the temperature that, when exceeded, caused an individual to become female. This term is not to be confused with pivotal temperature, which describes the temperature producing a 1 : 1 sex ratio of a clutch or population (Mrosovsky and Pieau 1991) rather than an attribute of an individual. For the purposes of this study, pivotal temperature is defined as the mean threshold temperature for the population. Within clutches (consisting of eight offspring), each individual's genetically inherited threshold temperature was compared to the nest temperature (T_{yj}) experienced by that clutch. If the nest temperature experienced by a clutch was higher than an individual's threshold temperature, the individual was recruited as a female into the adult population; otherwise it was recruited as a male (fig. 1).

Individual survivorship varied across two life stages: offspring survivorship to year 1 and adult survivorship. Survivorship rates for males and females were equal within all life stages. The breeding population consisted of all males of at least age 3 and all females of at least age 5, equal to estimated ages of first reproduction for painted turtles in the study population (F. J. Janzen, personal communication). Each female mated with a randomly selected male and produced one clutch of eggs per year. During years of female-biased sex ratios, a marriage function determined the fraction of females ($B[n_f]$) that bred during that year:

$$B(n_f) = \frac{2n_m}{n_m + n_f}, \tag{2}$$

where n_m is the number of males and n_f is the number of females in the breeding population. The harmonic mean was chosen because it is considered to be less problematic than using simply a female-dominant marriage function in two-sex models (Caswell and Weeks 1986; Caswell 1989). Although probably having no biological basis in turtles, this function was useful for the simulations because it prevented exploding population growth and crashing simulations during years of highly female-biased sex ratios. However, no qualitative differences in relative rates of evolution of nest-site choice and threshold temperatures, or the rate of sex ratio evolution, occurred whether or not the function was included in the simulations (C. L. Morjan, unpublished data).

Inheritance of Z *and* N. The mode of inheritance of threshold temperature (Z) and nest-site choice (N) is based on an earlier model of additive genetic variation for threshold temperatures by Bull and Bulmer (1989). Each trait consisted of five unlinked, biallelic loci, four of which affected the mean of the trait's expression and one that affected its variance. Alleles for loci affecting a trait's mean had an effect size of 1 or -1, and an individual's mean threshold

temperature ($\overline{Z}_i$) was offset from the population mean $\overline{Z}$ according to the sum of the allele values:

$$\overline{Z}_i = \overline{Z} + \mu_Z(l_{11} + l_{12} + l_{21} + \ldots + l_{42}), \quad (3)$$

where l_{jk} represents the value attributed to allele k at locus j. Alleles for the variance locus had an effect size of 1 or 0, and an individual's variance in expression of threshold temperature was calculated as

$$V_{Z_i} = \sigma^2_{aZ}(l_{51} + l_{52}) + \sigma^2_{bZ}[2 - (l_{51} + l_{52})], \quad (4)$$

which allowed 1's to represent the value of σ_a^2 and 0's to represent the value of σ_b^2. An individual's expressed threshold temperature was then calculated from a normal distribution of mean $\hat{Z}_i$ with variance $\hat{V}_{Z_i}$. Individual means and variances for nest-site choice (N_i) were also calculated similarly.

Alleles affecting the mean of a trait were allowed to mutate ± 1 value at a rate of 10^{-5} per allele per generation (following Bull and Bulmer 1989). In addition, three null loci affecting the mean of each trait, all originally set to 0 (i.e., initially having no effect on the mean), were allowed to mutate along with the four original alleles concerning a trait's mean, simulating added function and thereby allowing the mean of a trait to evolve outside of its initial phenotypic range if further mutations were acquired at these loci. Ultimately, mutations in this model turned out to be relatively unimportant given the number of generations that simulations were run.

Model Calibration. The following parameter estimates ($\pm$SE) for equation (1) were derived from a multiple linear regression of July air temperatures and nest microhabitat on nest temperatures: intercept ($\hat{\alpha}$): -1.49 ± 2.11; mean July air temperature ($\hat{\beta}$): 1.18 ± 0.09; average percentage of south and west vegetation ($\hat{\gamma}$): -0.035 ± 0.004. No interaction effect between climatic condition and vegetation cover on nest temperatures was present ($P = .75$). The parameters α, β, γ, and ε for equation (1) were chosen to be 0, 1.1, -0.035, and 0, respectively (with a normally distributed variance of 1.0 for ε), based on the derived estimates for α, β, and γ and how closely the modeled data fit actual data available from the study population for the ranges and distributions of nest temperatures.

Long-term thermal data reported from a weather station near the field site indicated a normal distribution of July temperatures for the past 50 yr ($\overline{X} \pm \text{SD} = 23.60° \pm 1.11°$C; Janzen 1994*a*). Based on these data, July temperature for each year was thus modeled as a random variable selected from a normal distribution centered at a mean of 23.6 with standard deviation of 1.11.

Adult survivorship varied between 85% and 90% per year for both sexes, which is within the estimates for adult painted turtles in the wild (Wilbur 1975; Mitchell 1988; Zweifel 1989; Iverson and Smith 1993). Survivorship decreased linearly within this range as density of the adult population increased, which kept population sizes stable over time. Offspring survivorship rate was calculated arbitrarily as 4% through incubation to age 1 (as a combination of clutch survivorship of 25% and recruitment rate of 16%), which provided a stable population size during constant climatic conditions. This estimate nevertheless falls into clutch and juvenile survivorship estimates for several *C. picta* populations (2%–10%; Gibbons 1968; Wilbur 1975; Zweifel 1989).

Distributions of the two traits from the model, N and Z, were compared to actual distributions of nest-site choice and thermal sensitivity of embryonic sex determination from the focal population and published literature. The values μ, σ_a^2, and σ_b^2 for threshold temperature (nest-site choice) were chosen to adequately capture known phenotypic ranges for the traits as well as among- and within-clutch (female) phenotypic variation observed for these traits. For threshold temperatures, the combination of these three values determined the range of temperatures that thresholds may vary (TRT). The values μ, σ_a^2, and σ_b^2, set at 0.18, 0.18, and 0.06, respectively, followed levels of among-clutch variation in sex ratio and TRT calculated from the focal population. When μ, σ_a^2, and σ_b^2 were set at 0.10, 0.08, and 0.02, respectively, they followed levels of among-clutch variation in sex ratio and TRT derived from the literature. Table 1 summarizes results from typical simulations of among-clutch variation in sex ratio for clutches across incubation temperatures when TRT = 2.23 and 1.41.

The values μ, σ_a^2, and σ_b^2 for nest-site choice, set at 4, 275, and 125, respectively, caused the distribution of vegetation cover over nests and repeatability of females to nest microhabitat to closely follow field-collected data. Mean repeatability ($\pm$SD) of nest-site choice was 0.23 ± 0.042 from 30 simulations of 180 females laying three nests each. This value falls near the range of repeatability estimates calculated for females laying three nests each at the field site (0.21; Janzen and Morjan 2001). Figure 2*A* and 2*B* illustrates the distribution of microhabitat for nests in a typical simulation and the distribution for nests in the field, respectively (data from Janzen and Morjan 2001).

Finally, annual offspring sex ratios as a function of yearly climate for a typical simulation are shown in figure 3. A typical simulation is defined as a simulation exhibiting parameters closest to the mean taken from 20–30 simulations. These tables and figures suggest that the model

Table 1: Offspring sex as a function of temperature from 12 clutches of *Chrysemys picta* eggs from Thomson, Illinois, and from typical simulations of two threshold temperature (TRT) parameter values (eight eggs per simulated clutch)

Experiment and effect	TRT ± SD	Likelihood ratio χ^2	df	*P*
Illinois ($n = 138$)	2.65 ± .67			
Clutch		29.28	11	.0021
Temperature		79.80	4	<.0001
Clutch × temperature		39.05	44	.68
Typical simulation ($n = 144$)	2.23 ± .35			
Clutch		26.64	11	.0052
Temperature		89.31	4	<.0001
Clutch × temperature		32.66	44	.89
Typical simulation ($n = 288$)	1.41 ± .16			
Clutch		52.5	23	.0004
Temperature		143.3	2	<.0001
Clutch × temperature		44.9	46	.52
Rhen and Lang 1998 ($n = 268$)	NA			
Clutch		53.7	23	.0003
Temperature		152.7	2	<.0001
Clutch × temperature		23.7	46	.98

Note: Likelihood ratio χ^2 values from logistic regression are provided. Data from *Chrysemys picta* (Rhen and Lang 1998) are provided for comparison; NA = not available.

adequately captured biologically relevant levels of variation for these two traits.

Simulations. For tests examining relative rates of change in allelic composition of threshold temperatures and nest-site choice, 30 trials simulating 1,000 yr each were run. In simulations involving climate change, a 1°C increase or decrease in mean climatic temperature gradually occurred after year 50. Between years 50 and 150, the mean temperature increased (or decreased) 0.01°C per year, until the mean temperature at year 150 was 1°C higher (or lower) than the original mean. The variance in climate remained the same during and after the changes. A 2°C change in this period of time often caused population extinctions, so changes greater than 1°C were not used in any analyses.

Changes in threshold temperature and nest-site choice were measured by allelic composition to allow both traits to be compared on the same scale. Allelic composition is simply the sum of the values for all loci concerning the mean expression of a trait in an individual (l_{jk}; eq. [3]). Changes in population mean allelic composition for a simulation were calculated by taking the difference in means between the end of the simulation and year 50 (the year immediately before climatic change). Changes in allelic composition (which were normally distributed) for the two traits were compared using paired *t*-tests.

In some species with TSD, females may conceivably return to nesting grounds as a result of natal imprinting rather than biparental genetic contribution (Freedberg and Wade 2001). The model was accordingly modified to address this scenario. Using the same initial settings, females again nested following the distribution observed at the actual site (fig. 2*A*, 2*B*). However, the mode of transmission of nest-site choice (eq. [3]) was changed in the model. In this case, offspring of these females nested in microhabitats with a mean consisting of their own respective natal microhabitat values, with a maternally inherited variance for nest-site choice as described in equation (4). All simulations were performed using Borland Delphi 4 (Inprise).

Calculating Heritabilities. Heritabilities (h^2) for nest-site choice and nest temperatures were calculated by using mother-daughter regression, and heritability for threshold temperature was calculated by mother-offspring regression for each of 20 populations of 617–956 individuals existing at the end of 50-yr simulations of no climate change.

The "effective heritabilities" for both threshold temperatures (effective h_Z^2) and nest temperatures (effective h_T^2) were calculated by multiplying their respective heritabilities by a relative variance term (Bull et al. 1982*a*; Bulmer and Bull 1982). The relative variance term accounts for the relative proportion of selection occurring on nest or threshold temperatures through offspring sex ratios and affects the expected rate of change for each character (Bull et al. 1982*a*). The effective heritability of threshold temperature is thus

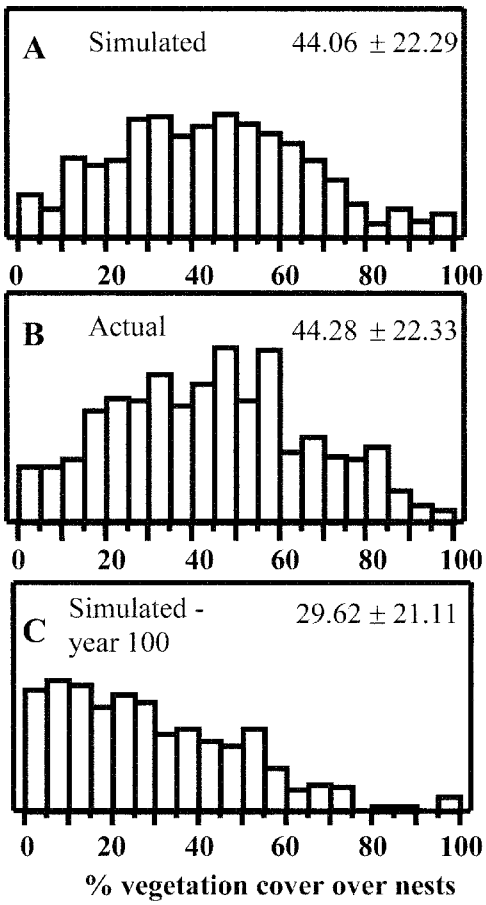

Figure 2: Distributions of percentage of vegetation cover over nests ($\bar{X} \pm$ SD) from (*A*) a typical 3-yr simulation ($N = 540$), (*B*) for all *Chrysemys picta* nests from Thomson, Illinois, from 1995 to 1999 ($N = 879$), and (*C*) after 100 yr during a typical simulation involving natal imprinting to nest microhabitats.

$$\text{effective } h_Z^2 = h_Z^2 \frac{\sigma_Z^2}{\sigma_Z^2 + \sigma_T^2}, \tag{5}$$

where σ_Z^2 is the phenotypic variance for threshold temperature and σ_T^2 is the phenotypic variance in nest temperatures. Similarly,

$$\text{effective } h_T^2 = h_T^2 \frac{\sigma_T^2}{\sigma_T^2 + \sigma_Z^2}. \tag{6}$$

Effective heritabilities for nest temperature and threshold temperature are used because offspring sex is affected directly by both factors (fig. 1). Calculating the effective heritability of nest-site choice on sex ratio is not appropriate because it affects sex indirectly through nest temperature.

Repeatability (R) for nest-site choice in terms of vegetation cover was calculated by first conducting a one-way ANOVA and then using the within- and among-female mean squares to calculate R (Lessells and Boag 1987; Janzen and Morjan 2001).

Results

Calculating TRT for Focal Population and from Literature

Both clutch and temperature significantly affected offspring sex ratios for Illinois *Chrysemys picta* eggs incubated in the laboratory (table 1). The TRT ($\pm$SD) for this population was calculated to be 2.65 ± 0.67. The calculated TRTs for Wisconsin and Tennessee populations of *C. picta* are 1.29 ± 0.036 and 1.23 ± 0.25, respectively. These values indicated a significantly narrower transitional range in temperatures producing mixed sex ratios for Wisconsin and Tennessee populations than for the focal population (*t*-tests: Illinois-Wisconsin: $t = 4.50$, df = 4, $P < .01$; Illinois-Tennessee: $t = 4.45$, df = 5, $P < .001$). Modeling a relatively narrower TRT = 1.41 ± 0.16 provided levels of among-clutch variation in sex ratios similar to those in a published study from a North Dakota population of *C. picta* (Rhen and Lang 1998; table 1). To provide the most conservative comparisons between relative rates of change in threshold temperatures versus nest-site choice, all further results are reported from models using the TRT of

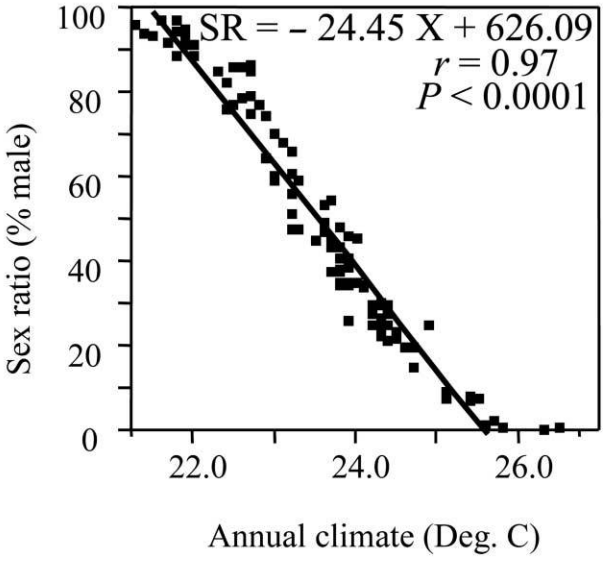

Figure 3: Results from a typical simulation of annual offspring sex ratio as a function of yearly climatic condition. The linear regression of annual cohort sex ratio (% male) as a function of mean July air temperature reported for this population of *Chrysemys picta* is $Y = -25.59$ (mean July air temperature) + 651.25, $r = 0.91$, $P = .03$ (Janzen 1994*a*).

1.41 unless indicated otherwise. However, results from simulations with a wider TRT (2.23; table 1) are also shown for two climate change simulations to compare the effects of these two values of TRT on evolutionary change in sex ratios.

Relative Rates of Evolution for Threshold Temperature and Nest-Site Choice

In simulations with no climate changes, adult sex ratios remained stable over time with a mean of 0.5 (fig. 4*A*). Figure 4*B* depicts sample simulations plotting mean allelic composition for both traits over time. Higher values for allelic composition are associated with a higher likelihood of producing males, through either increased vegetation cover causing cooler nests or higher threshold temperatures. In several simulations, a change in mean allelic composition for threshold temperature was accompanied by a corresponding change in nest-site choice in the opposite direction as a result of Fisherian sex-ratio selection. For example, if females nested under increased vegetation cover over time because of genetic drift, threshold temperatures "compensated" by decreasing the likelihood of producing males at cooler nest temperatures. Indeed, a negative correlation for the two traits existed across populations at the end of the simulations ($n = 30$ simulations, $r = -0.72$, $P < .0001$). However, no genetic correlation was found for the two traits within individuals (r ranged from 0.1418 to −0.1933 for 100 randomly sampled individuals from each of the 30 simulations, all NS). Changes in mean allelic compositions for nest-site choice and mean threshold temperatures did not differ significantly from 0 (two-tailed t-test; nest-site choice: $t = 0.168$, df = 29, $P = .87$; threshold temperature: $t = 0.0049$, df = 29, $P = 1.0$) or from each other (paired t-test, $t = 0.096$, df = 29, $P = .92$).

In simulations involving an increase in temperature of 1°C, adult sex ratios initially became female biased and then gradually approached 1 : 1 (fig. 5*A*). Population sex ratios remained biased for several hundred years under both TRT estimates (1.41 and 2.23); however, adult sex ratios approached 1 : 1 more rapidly under the wider TRT (fig. 5*A*). This return to 1 : 1 sex ratios is due to Fisherian sex-ratio selection for increased threshold temperatures and for females to place nests under higher vegetation cover, both of which bias offspring sex ratios toward males (fig. 5*C*). Both threshold temperature and nest-site choice exhibited a change in mean allelic composition significantly greater than 0 (two-tailed t-test; nest-site choice: $t = 22.25$, df = 29, $P < .0001$; threshold temperature:

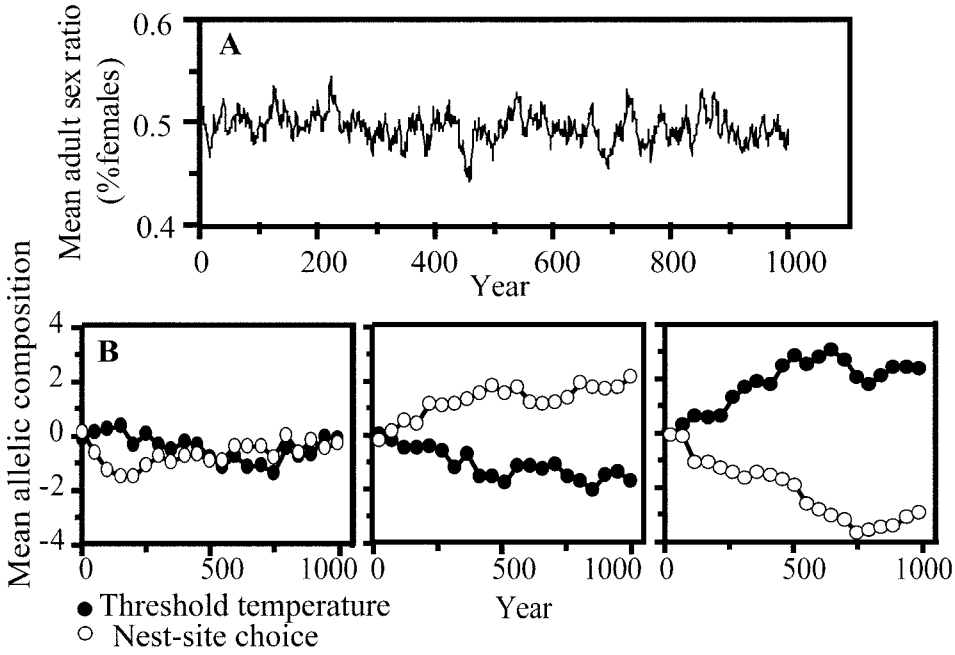

Figure 4: Results from sample simulations of *Chrysemys picta* populations without climatic change; threshold temperature (TRT) = 1.21. *A*, Mean population sex ratios. Standard errors for the mean are ±1%. *B*, Examples of temporal changes in mean allelic composition in the populations for threshold temperature and nest-site choice for vegetation cover over nests. Increasing values of allelic composition indicate an increase in the population mean for that trait.

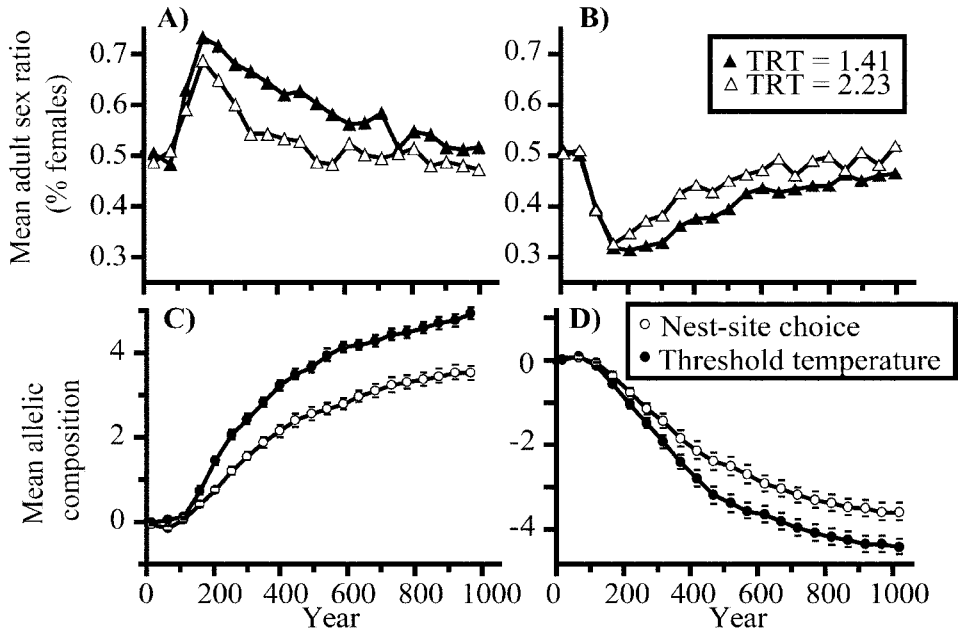

Figure 5: Mean adult sex ratios and changes in allelic composition in the populations by year for (*A*, *C*) a 1°C increase in climate and (*B*, *D*) a 1°C decrease in climate. In the top panels, filled and open triangles represent adult sex ratio when threshold temperature (*TRT*) = 1.41 and 2.23, respectively. Standard errors for sex ratio at TRT = 1.41 and TRT = 2.23 are ±1.0% and ±0.015%, respectively. Mean allelic composition for threshold temperature (*filled circles*; when TRT = 1.41, most conservative estimate) and nest-site choice (*open circles*) are shown in the bottom panels, with standard errors represented by bars.

$t = 36.75$, df = 29, $P < .0001$). However, threshold temperature experienced a relatively greater change than did nest-site choice (two-tailed paired *t*-test: $t = 4.74$, df = 29, $P < .0001$).

Similarly, a decrease in annual temperature of 1°C over 100 yr caused sex ratios to become initially male biased and eventually return to 1 : 1 (fig. 5*B*). In this case, several populations went extinct, so a second set of 30 simulations was run and the data combined to analyze the surviving populations. Out of the 60 simulations, 45 populations survived. Both traits exhibited a change in mean allelic composition in the negative direction (two-tailed *t*-test: nest-site choice: $t = -17.91$, df = 44, $P < .0001$; threshold temperature: $t = -25.81$, df = 44, $P < .0001$; fig. 5*D*). Threshold temperature again exhibited a relatively greater change than did nest-site choice overall (two-tailed paired *t*-test: $t = 2.53$, df = 44, $P = .015$). Simulations using TRT = 2.23 exhibited three differences from those using TRT = 1.41: sex ratios approached 1 : 1 more rapidly (fig. 5), threshold temperatures evolved even more rapidly than did nest-site choice (see discussion on heritabilities), and no extinctions occurred during a 1°C cooling in climate (0 of 30 vs. 15 of 60; Fisher's exact test, $P = .0018$).

Heritabilities for nest-site choice, nest temperatures, and threshold temperatures for the additive genetic model of inheritance are summarized in table 2. Although both nest-site choice and threshold temperature exhibited substantial heritabilities, heritability for nest temperatures as well as effective heritabilities for all traits were quite low.

Effects of Natal Imprinting on Nest-Site Choice

In simulations involving natal imprinting with no climatic change, female-biased sex ratios resulted (fig. 6*A*). Micro-

Table 2: Heritabilities (h^2), phenotypic variances (used to calculate effective heritabilities), and effective heritabilities for nest-site choice, nest temperatures, and threshold temperatures (TRT) for 20 simulations of 617–956 parent-offspring pairs at the end of 50 yr

Trait	h^2 ± SD	Phenotypic variance	Effective h^2
Nest-site choice	.22 ± .086	…	…
Nest temperature	.044 ± .20	2.59	.041
Threshold temperature:			
TRT = 1.41	.44 ± .089	.17	.026
TRT = 2.23	.50 ± .058	.45	.074

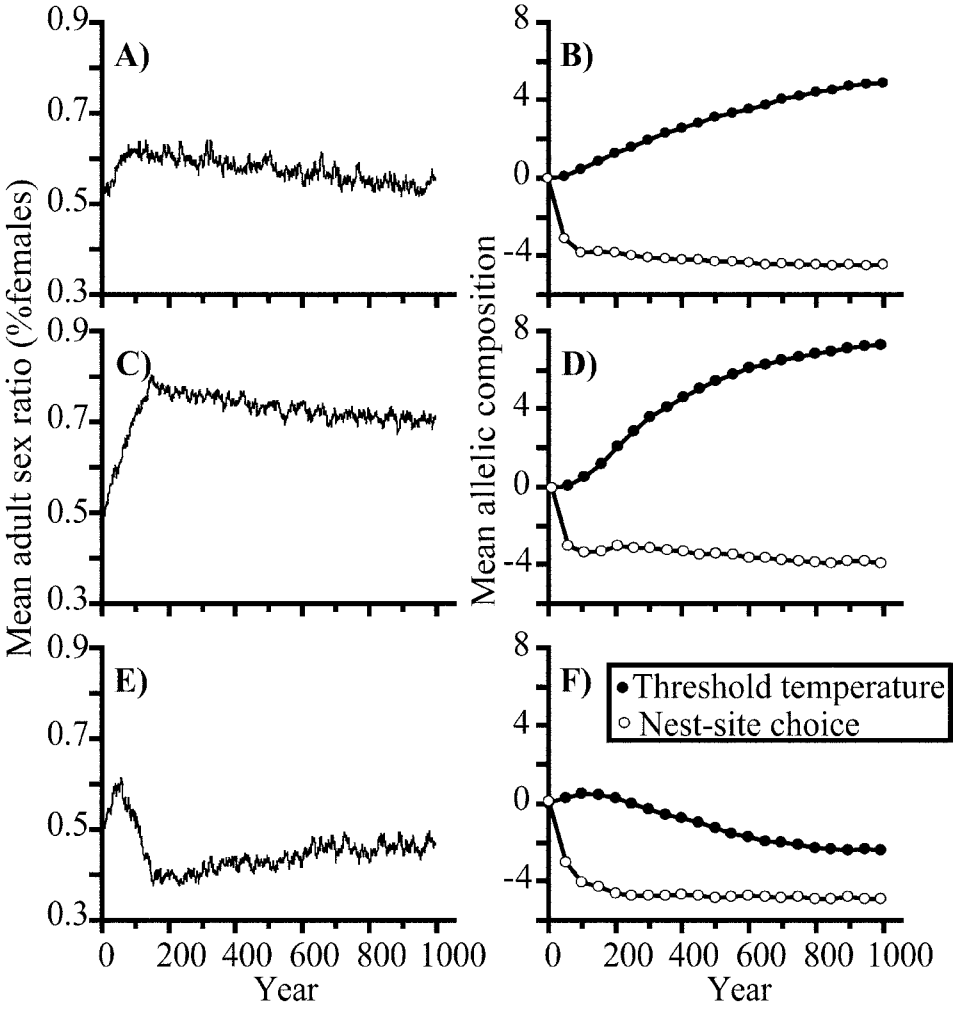

Figure 6: Changes in three key parameters in simulations with natal imprinting to nests during (*A*, *B*) no climatic change, (*C*, *D*) a 1°C increase in climate, and (*E*, *F*) a 1°C decrease in climate. The left panels represent changes in mean adult sex ratios (SE $< \pm 0.02$). The right panels show corresponding changes in mean allelic composition for threshold temperatures (TRT = 1.41; *filled circles*) and nest-site choice (*open circles*) for each of the climate scenarios shown on the left, with standard errors ± 0.18 and ± 0.09, respectively.

habitat selection rapidly and consistently changed to sites with lower vegetation cover (fig. 6*B*). The greatest change in nest-site choice typically occurred within the first 100 yr and subsequently reached equilibrium because vegetation cover over nests could not be <0. Figure 2*C* shows the distribution of vegetation cover over nests after 100 yr during a typical simulation of natal imprinting to nests with no climate change. Although threshold temperatures subsequently increased to compensate for the female-biased sex ratios (fig. 6*B*), sex ratios even at the end of the 1,000-yr simulations remained significantly female biased ($\overline{X} \pm \text{SD} = 0.560 \pm 0.75$; hypothesized value = 0.5, $t = 4.37$, df = 29, $P < .0001$). However, sex ratios slowly but steadily approached 0.50 throughout the simulations, regardless of climate scenario (fig. 6).

Nest-site choice evolved toward microenvironments likely to produce females regardless of whether the populations experienced an increase or decrease in climate (fig. 6). Females nested in sites with less vegetation cover, sites that are likely to produce females, even in a climatic warming scenario that produced an excess of females (fig. 6*D*). The direction of climate change caused relatively small alterations in nest-site choice compared to changes in threshold temperature (fig. 6*D*, 6*F*). In contrast to additive genetic inheritance of nest microhabitat, no populations experienced extinction during a 1°C decrease in

climatic conditions, probably because sex ratios had already become female biased before climatic cooling occurred (fig. 6*E*).

Discussion

Evolution of Maternal versus Offspring Traits Affecting Sex

Although many reptilian taxa with TSD exhibit substantial variation in sex ratios among families and populations (Bull et al. 1982*a*, 1982*b*; Janzen 1992; Vogt and Flores-Villela 1992; Ewert et al. 1994; Rhen and Lang 1998; Bowden et al. 2000), maternal choice of nest sites is generally thought to be more likely to respond to sex ratio selection (Bull et al. 1982*a*, 1982*b*; Bulmer and Bull 1982). This reasoning is because thermal ranges of natural nests greatly exceed the thermal range where genetic variation in sex ratios is expressed, therefore providing relatively little opportunity for selection to act on this genetic variation. Indeed, low effective heritabilities for offspring sex ratio are expected in the field (0.03–0.06: Bull et al. 1982*a*; Bulmer and Bull 1982; Janzen 1992). Consequently, variation in thermal choice of nest sites has been thought to be more likely to evolve in the maintenance of sex ratios (Bull et al. 1982*a*, 1982*b*; Bulmer and Bull 1982).

This study demonstrates that threshold temperatures are more likely to respond to sex ratio selection than female nest-site choice, given observed levels of variation for these traits in a population of *Chrysemys picta* with TSD. These results seem paradoxical in the sense that variation across nest microenvironments within a year varied on average by 6.4°C because of variation in nest-site selection by females, whereas threshold temperatures varied by only half this amount, at best. Both traits may also have limited evolutionary potential in response to rapid climatic changes in this population.

Nest-site choice evolved more slowly relative to threshold temperature for two main reasons: a low effective heritability for nest temperatures and the maternal expression of nest-site choice. First, heritability for threshold temperature was higher than the heritability for nest-site choice (table 2), which may be expected considering the high heritabilities estimated for the former trait in laboratory experiments using freshwater turtles ($h^2 = 0.82$ [Bull et al. 1982*a*]; $h^2 = 0.26$–1 [Janzen 1992]). The two studies to date evaluating the upper limit of the heritability of nest-site choice in reptiles with TSD have both estimated values close to 0.20 (Bull et al. 1988; Janzen and Morjan 2001). On the surface, nest-site choice appears likely to respond more rapidly to selection because of its higher heritability than effective heritabilities for threshold temperatures (~0.20 compared to ~0.06). However, heritability in nest-site choice translated into an extremely low heritability for nest temperatures (0.04). Nest temperatures of parents and offspring exhibited little resemblance to each other because of the overriding effect of climatic variation among years. Additional simulations of my model indicated that the repeatability for individual females in terms of vegetation cover over nests would need to approach 0.60 to evolve at a rate similar to the more conservative estimate (TRT = 1.41) for threshold temperatures (data not shown). Even by repeatedly nesting in similar microhabitats, females may therefore have relatively little control over choosing thermal environments to lay their eggs. Similarly, Valenzuela and Janzen (2001) did not observe repeatability in clutch sex ratios for individual female *C. picta* across five nesting seasons, although individual females in this population repeatedly nested in microhabitat qualities correlated with nest temperatures.

The second reason that threshold temperature evolved more rapidly than did nest-site choice is because nest-site choice is a maternal effect. The significance of this effect can be demonstrated in my model by allowing nest microhabitat for an egg to be determined by an offspring's own genotype rather than its mother's (which is unlikely in nature but used to demonstrate this particular point). In this case, nest-site choice evolved more rapidly than did threshold temperatures when climate increased 1°C (two-tailed paired *t*-test, nest-site choice: 4.76 ± 1.04, threshold temperature: 3.88 ± 0.87, $t = 2.77$, df = 29, $P < .001$). Maternal nest-site choice experiences indirect selection because the phenotype on which selection acts directly (sex of the individual) is not expressed by the mother but by her offspring. These offspring do not carry the genotype for microhabitat selection that affects their sex but rather a correlated genotype for microhabitat selection through Mendelian inheritance. Since selection acts directly on offspring sex, the evolution of thermal sensitivity of offspring sex depends on the extent to which offspring phenotypes and genotypes for this trait are correlated, whereas the evolution of nest-site choice depends on the extent to which offspring and maternal genotypes for this trait are correlated. Such dynamics have also been demonstrated to affect epistatic interactions between maternal and offspring traits (Wolf 2000).

Effects of Natal Imprinting

In sea turtles, mitochondrial haplotypes specific to nesting beaches or even sections of these beaches suggest that females inherit nest sites from their mothers, either genetically or through imprinting (Bowen et al. 1992; Peare and Parker 1996; Bass et al. 1996). In populations exhibiting female nest-site philopatry, nesting frequency is expected

to increase in sites likely to produce females, causing a runaway process resulting in female-biased sex ratios (Bull 1980; Reinhold 1998; Freedberg and Wade 2001). This phenomenon is expected to occur because female-producing sites are the sites to which many individuals return to nest in subsequent generations, whereas sites producing mostly males produce few females that return to nest. My model confirms that female-biased sex ratios may arise under philopatry without climate change. Most important, it shows that nest-site choice responded minimally during climate change scenarios and in fact was maladaptive during climatic warming, with females laying eggs in female-producing environments.

In the focal population, nest-site fidelity to specific geographic areas occurs within females but at a scale that is too large to explain repeatability in overstory vegetation cover over nests (Janzen and Morjan 2001; Valenzuela and Janzen 2001). Females repeatedly nest within specific geographic areas, but repeatability of nesting to specific microhabitats is not due to geographic location because these areas contain a high level of microhabitat heterogeneity within them. Although there are insufficient data to support or refute whether natal philopatry to nest microhabitat occurs in this population, my simulations predict that under natal philopatry the current distribution of vegetation cover over nests should change rapidly over a relatively short time span in this population of painted turtles (fig. 2*C*), along with a rapid change toward female-biased sex ratios (fig. 6*A*). The observations that the distribution of vegetation cover over nests has favored intermediate sites for the past 13 yr of study (two to three generations) and that female-biased sex ratios are not evident in this population (Janzen 1994*a*; F. J. Janzen, personal communication) suggest against a possible mechanism of natal imprinting to nest vegetation cover affecting sex ratios in this population.

Implications for Variation in Natural Populations

The predictions from my model rely on the assumption that there is an additive genetic basis for the inheritance of traits affecting offspring sex ratios. Past studies have generally assumed an additive genetic basis for the evolutionary potential or dynamics of nest-site choice and threshold temperatures (Bull et al. 1982*a*; Bulmer and Bull 1982; Janzen 1992; Rhen and Lang 1998; but see Bowden et al. 2000; Freedberg and Wade 2001). Similarly, threshold traits typically have an underlying additive genetic component to them as well as environmental sensitivity for their expression (reviewed in Roff 1996).

Maternal effects and dominance variance may also affect among-family phenotypic variance in these traits (Falconer and Mackay 1996). Although evaluations of the evolutionary potential for among-clutch variation in sex ratios have assumed no maternal effects (Bull et al. 1982*a*; Janzen 1992; Rhen and Lang 1998), recent evidence suggests that clutch sex ratios are affected by yolk steroid hormone concentrations (Janzen et al. 1998) that vary seasonally (Bowden et al. 2000). Sex ratio variation across clutches may be enhanced by this maternal component, possibly reducing heritability estimates. For this reason, my model may provide an upper estimate of the evolutionary potential for sex ratios in this population, which is nevertheless low.

However, the model may provide a lower estimate of the evolutionary potential for offspring sex ratios if genotype × environment interactions exist in *C. picta*. Rhen and Lang (1998) suggested that genotype × environment interactions increase the evolutionary potential of sex ratio in this system by allowing changes in the shape of the response curve of sex ratio to temperature, rather than simply shifting the response curve to the left or right, in response to climate change. Although the transition range producing mixed sex ratios is wider in the Illinois population than in the two other populations studied, no genotype × environment interactions for clutch sex ratios have yet been found within populations of *C. picta* (Rhen and Lang 1998; table 1). However, in my study, eggs would probably need to be incubated at fewer than five temperatures to detect such an interaction since clutch sizes were small (12 eggs per clutch on average in this population). It could be interpreted that variation in TRT detected among *C. picta* populations in this study suggests a genotype × environment interaction for offspring sex ratio. However, variation in timing of nesting by females, which is correlated with yolk steroid hormones affecting offspring sex (Bowden et al. 2000), and clutch effects by females within populations were not taken into account when calculating TRT values. Future studies controlling for these effects in a common-garden environment could provide a clearer understanding of potential genotype × environment interactions in *C. picta*.

My model also predicts that thermal sensitivity of offspring sex ratios is likely to coevolve with patterns of genetically inherited nest-site choice under stable climatic conditions (fig. 4*B*). Latitudinal clines in pivotal temperatures, expected to compensate for climatic differences across populations, are generally lacking in turtles (Bull et al. 1982*b*; Mrosovsky 1988; Vogt and Flores-Villela 1992; Ewert et al. 1994). Although geographic variation in nest-site choice mitigating nest temperatures among populations may explain this lack of a trend (Bull et al. 1982*b*; Ewert et al. 1994), nesting patterns may primarily reflect maximizing egg development or survival rather than adaptively adjusting sex ratios (Ewert et al. 1994; Morjan 2003).

One major assumption and potential caveat of this

model is that nesting dates were assumed to be fixed in the population. Changes in nesting dates could mitigate effects of climatic changes on nest temperatures; perhaps females could nest earlier in the season during hotter years. Nesting phenology changes with climatic condition in some populations of birds (Dunn and Winkler 1999) but remains undocumented in reptiles except for some casual observations. This assumption of fixed nesting dates in part follows implicit assumptions in past studies for the evolution of sex ratios through maternal choice of nest site (Bull et al. 1982*a*; Bulmer and Bull 1982; Janzen 1992; Ewert et al. 1994; but see Bull et al. 1982*b* and Vogt and Bull 1982). Furthermore, variation in nesting dates lacks empirical evidence in *Chrysemys picta bellii* both within the Illinois population (Janzen 1994*a*) and across their range (Christiansen and Moll 1973; Morjan 2003). No repeatability of individual females for nesting dates or relative order of nesting has been found for females in this population (F. J. Janzen and C. L. Morjan, unpublished data). *Chrysemys picta bellii* in New Mexico, which typically experience July temperatures >2°C higher than their Illinois conspecifics, also nest at almost exactly the same time as the Illinois population (Morjan 2003). Latitudinal trends in nesting phenology may be present in other North American freshwater turtles (Moll 1979; Vogt and Bull 1982), but further investigation is needed to make strong conclusions for chelonian taxa.

Evolutionary Potential of Sex Ratio

In the near future, global climatic temperatures are expected to increase as a result of human activities by 1°–5°C per 100 yr (Schneider 1989; Root and Schneider 1993; O'Neill et al. 2001). Recently derived models predict an increase of about 1.5°C within the next 50 yr in North America (Zwiers 2002). Rates of climatic changes at the lower end of this range were modeled because many simulations involving greater changes resulted in high levels of extinction. Although extinction rates under these scenarios were sensitive to even small changes in density-dependence functions, TRT, population size, and survivorship in most life stages, the results for relative rates of evolution of nest-site choice and threshold temperatures remained robust to changes in all of these factors. Consequently, the implications for this model concern the relative rates of evolution for nest-site choice and pivotal temperatures; more data are required to accurately model predicted effects of climate change on population dynamics or extinction rates. My model also does not include Allee effects of mate search strategies (Berec et al. 2001) or metapopulation dynamics involving various nesting beaches with different microhabitat-temperature functions, both of which may also be critical for projections of extinction risk.

Adaptive mechanisms counteracting Fisherian sex ratio selection in reptiles have received little consistent empirical support (reviewed in Shine 1999). On the one hand, sex ratios have responded rapidly to Fisherian selection in silverside fish (six to eight generations; Conover and Van Voorhees 1990) and in *Drosophila* hybrids (16 generations; Blows et al. 1999). On the other hand, Fisherian selection was expected to act quite slowly (330 generations) in an experimental population of *Drosophila mediopunctata* and was expected to evolve slowly in general (Carvalho et al. 1998). My model suggests that sex ratios in reptiles with TSD should evolve slowly in response to expected climate changes (~550 yr; fig. 5). However, in terms of generation time, expected rates of evolution may fall within the ranges of empirical studies in other organisms (50–100 generations on average). Nevertheless, projected climate changes may be quite rapid compared to the expected time required to restore sex ratios in turtles.

Evolutionary dynamics of maternal effects may include nonintuitive responses such as time lags, temporary maladaptive responses to selection, or runaway processes (Kirkpatrick and Lande 1989; Wade 1998). Furthermore, it is important to quantify the amount that environmental variation modifies maternal effects (reviewed in Rossiter 1998), as demonstrated by effects of yearly climate in this scenario. Besides modifying their nesting behavior or threshold temperatures, reptiles with TSD are thought to face three alternative responses to climate change: modify their geographic ranges, change to genotypic sex determination, or face extinction (Janzen and Paukstis 1991*a*). In light of the predicted outcomes by my simulation model, evolutionary responses in these reptiles may be relatively ineffective in response to sex-ratio perturbation caused by rapid climate change. Ecological responses, such as plasticity in nesting phenology or habitat change, may instead be a more rapid outcome from such climate change scenarios.

Acknowledgments

Protocol for the collection and incubation of eggs and hatching and sexing of hatchling turtles was approved by the Committee for Care and Use of Animals in Research at Iowa State University (1-8-3785-1-J). Turtle eggs were collected under authority of Special Use Permit 98006 from the U.S. Fish and Wildlife Service and scientific permit NH98.0099 from the Illinois Department of Natural Resources. Empirical information used to parameterize the models was collected under National Science Foundation (NSF) grant DEB-9629529 to F. J. Janzen. This research was supported in part by a predoctoral fellowship from

the NSF and a Leopold Brown Trust fellowship from Iowa State University. Special thanks to R. Bowden, W. R. Clark, F. J. Janzen, G. Naylor, E. Pollak, N. Valenzuela, and C. Vleck for insightful discussions and helpful comments on the manuscript; D. Adams, C. Eckerman, and O. Fedrigo for programming advice; and to all the current and past members of Janzen Lab turtle camp crew for collecting the field data to make this research possible.

Literature Cited

Arnold, S. J. 1994. Multivariate inheritance and evolution: a review of concepts. Pages 17–48 *in* C. R. B. Boake, ed. Quantitative genetic studies of behavioral evolution. University of Chicago Press, Chicago.

Bass, A. L., D. A. Good, K. A. Bjorndal, J. I. Richardson, Z.-M. Hillis, J. A. Horrocks, and B. W. Bowen. 1996. Testing models of female reproductive migratory behaviour and population structure in the Caribbean hawksbill turtle, *Eretmochelys imbricata*, with mtDNA sequences. Molecular Ecology 5:321–328.

Berec, L., D. S. Boukal, and M. Berec. 2001. Linking the Allee effect, sexual reproduction, and temperature-dependent sex determination via spatial dynamics. American Naturalist 157:217–230.

Blows, M. W., D. Berrigan, and G. W. Gilchrist. 1999. Rapid evolution towards equal sex ratios in a system with heterogamety. Evolutionary Ecology Research 1: 277–283.

Boake, C. R. B. 1989. Repeatability: its role in evolutionary studies of mating behavior. Evolutionary Ecology 3: 173–182.

Bowden, R. M., M. A. Ewert, and C. E. Nelson. 2000. Environmental sex determination in a reptile varies seasonally and with yolk hormones. Proceedings of the Royal Society of London B, Biological Sciences 267: 1745–1749.

Bowen, B. W., A. B. Meylan, J. P. Ross, C. J. Limpus, G. H. Balazs, and J. C. Avise. 1992. Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evolution 46:865–881.

Bragg, W. K., J. D. Fawcett, T. B. Bragg, and B. E. Viets. 2000. Nest-site selection in two eublepharid gecko species with temperature-dependent sex determination and one with genotypic sex determination. Biological Journal of the Linnean Society 69:319–332.

Bull, J. J. 1980. Sex determination in reptiles. Quarterly Review of Biology 55:3–21.

———. 1983. Evolution of sex determining mechanisms. Benjamin/Cummings, Menlo Park, Calif.

———. 1985. Sex ratio and nest temperature in turtles: comparing field and laboratory data. Ecology 66: 1115–1122.

Bull, J. J., and M. G. Bulmer. 1989. Longevity enhances selection of environmental sex determination. Heredity 63:315–320.

Bull, J. J., R. C. Vogt, and M. G. Bulmer. 1982*a*. Heritability of sex ratio in turtles with environmental sex determination. Evolution 36:333–341.

Bull, J. J., R. C. Vogt, and C. J. McCoy. 1982*b*. Sex determining temperatures in turtles: a geographic comparison. Evolution 36:326–332.

Bull, J. J., W. H. N. Gutzke, and M. G. Bulmer. 1988. Nest choice in a captive lizard with temperature-dependent sex determination. Journal of Evolutionary Biology 2: 177–184.

Bulmer, M. G., and J. J. Bull. 1982. Models of polygenic sex determination and sex ratio control. Evolution 36: 13–26.

Carvalho, A. B., M. C. Sampaio, F. R. Varandas, and L. B. Klaczko. 1998. An experimental demonstration of Fisher's principle: evolution of sexual proportion by natural selection. Genetics 148:719–731.

Caswell, H. 1989. Two-sex models. Sinauer, Sunderland, Mass.

Caswell, H., and D. E. Weeks. 1986. Two-sex models: chaos, extinction, and other dynamic consequences of sex. American Naturalist 128:707–735.

Chevalier, J., M. H. Godfrey, and M. Girondot. 1999. Significant difference of temperature-dependent sex determination between French Guiana (Atlantic) and Playa Grande (Costa-Rica, Pacific) leatherbacks (*Dermochelys coriacea*). Annales des Sciences Naturelles, Zoologie et Biologie Animale 20:147–152.

Christiansen, J. L., and E. O. Moll. 1973. Latitudinal reproductive variation within a single subspecies of painted turtle, *Chrysemys picta bellii*. Herpetologica 29: 152–163.

Conover, D. O., and D. A. Van Voorhees. 1990. Evolution of a balanced sex ratio by frequency-dependent selection in a fish. Science (Washington, D.C.) 250:1556–1558.

Dunn, P. O., and D. W. Winkler. 1999. Climate change has affected the breeding date of tree swallows throughout North America. Proceedings of the Royal Society of London B, Biological Sciences 266:2487–2490.

Ernst, C. H., J. E. Lovich, and R. W. Barbour. 1994. Turtles of the United States and Canada. Smithsonian Institution, Washington, D.C.

Ewert, M. A., and C. E. Nelson. 1991. Sex determination in turtles: diverse patterns and some possible adaptive values. Copeia 1991:50–69.

Ewert, M. A., D. R. Jackson, and C. E. Nelson. 1994. Patterns of temperature-dependent sex determination in turtles. Journal of Experimental Zoology 270:3–15.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. Longman, Essex.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon, Oxford.

Freedberg, S., and M. J. Wade. 2001. Cultural inheritance as a mechanism for population sex-ratio bias in reptiles. Evolution 55:1049–1055.

Gibbons, J. W. 1968. Population structure and survivorship in the painted turtle, *Chrysemys picta*. Copeia 1968: 260–268.

Girondot, M. 1999. Statistical description of temperature-dependent sex determination using maximum likelihood. Evolutionary Ecology Research 1:479–486.

Iverson, J. B., and G. R. Smith. 1993. Reproductive ecology of the painted turtle (*Chrysemys picta*) in the Nebraska sandhills and across its range. Copeia 1993:1–21.

Janzen, F. J. 1992. Heritable variation for sex ratio under environmental sex determination in the common snapping turtle (*Chelydra serpentina*). Genetics 131:155–161.

———. 1994*a*. Climate change and temperature-dependent sex determination in reptiles. Proceedings of the National Academy of Sciences of the USA 91: 7487–7490.

———. 1994*b*. Vegetational cover predicts the sex ratio of hatchling turtles in natural nests. Ecology 75: 1593–1599.

Janzen, F. J., and C. L. Morjan. 2001. Repeatability of microenvironment-specific nesting behaviour in a turtle with environmental sex determination. Animal Behaviour 62:73–82.

Janzen, F. J., and G. L. Paukstis. 1988. Environmental sex determination in reptiles. Nature 332:790.

———. 1991*a*. Environmental sex determination in reptiles: ecology, evolution, and experimental design. Quarterly Review of Biology 66:149–179.

———. 1991*b*. A preliminary test of the adaptive significance of environmental sex determination in reptiles. Evolution 45:435–440.

Janzen, F. J., M. E. Wilson, J. K. Tucker, and S. P. Ford. 1998. Endogenous yolk steroid hormones in turtles with different sex-determining mechanisms. General and Comparative Endocrinology 111:306–317.

Kirkpatrick, M., and R. Lande. 1989. The evolution of maternal characters. Evolution 43:485–503.

Lessells, C. M., and P. T. Boag. 1987. Unrepeatable repeatabilities: a common mistake. Auk 104:116–121.

Mitchell, J. C. 1988. Population ecology and life histories of the freshwater turtles *Chrysemys picta* and *Sternotherus odoratus* in an urban lake. Herpetological Monographs 2:40–61.

Moll, E. O. 1979. Reproductive cycles and adaptations. Pages 305–331 *in* M. Harless and H. Morlock, eds. Turtles: perspectives and research. Wiley, New York.

Morjan, C. L. 2002. Temperature-dependent sex determination and the evolutionary potential for sex ratio in the painted turtle, *Chrysemys picta*. Ph.D. diss. Iowa State University, Ames. 214 pp.

———. 2003. Variation in nesting patterns affecting nest temperatures in two populations of painted turtles (*Chrysemys picta*) with temperature-dependent sex determination. Behavioral Ecology and Sociobiology 53: 254–261.

Morjan, C. L., and F. J. Janzen. 2003. Nest temperature is not related to egg size in a turtle with temperature-dependent sex determination. Copeia (in press).

Mrosovsky, N. 1988. Pivotal temperatures for loggerhead turtles (*Caretta caretta*) from northern and southern nesting beaches. Canadian Journal of Zoology 66: 661–669.

Mrosovsky, N., and C. Pieau. 1991. Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles. Amphibia-Reptilia 12:169–179.

O'Neill, B. C., F. L. MacKellar, and W. Lutz. 2001. Population and climate change. Cambridge University Press, Cambridge.

Peare, T., and P. G. Parker. 1996. Local genetic structure within two rookeries of *Chelonia mydas* (the green turtle). Heredity 77:619–628.

Reinhold, K. 1998. Nest-site philopatry and selection for environmental sex determination. Evolutionary Ecology 12:245–250.

Rhen, T., and J. W. Lang. 1998. Among-family variation for environmental sex determination in reptiles. Evolution 52:1514–1520.

Roff, D. A. 1996. The evolution of threshold traits in animals. Quarterly Review of Biology 71:3–35.

Root, T. L., and S. H. Schneider. 1993. Can large-scale climatic models be linked with multiscale ecological studies? Conservation Biology 7:256–270.

Rossiter, M. 1998. The role of environmental variation in parental effects expression. Pages 112–134 *in* T. A. Mousseau and C. W. Fox, eds. Maternal effects as adaptations. Oxford University Press, Oxford.

Schneider, S. H. 1989. The greenhouse effect: science and policy. Science (Washington, D.C.) 243:771–781.

Shine, R. 1999. Why is sex determined by nest temperature in many reptiles? Trends in Ecology & Evolution 14: 186–189.

Valenzuela, N., and F. J. Janzen. 2001. Nest-site philopatry and the evolution of temperature-dependent sex determination. Evolutionary Ecology Research 3:1–17.

Vogt, R. C., and J. J. Bull. 1982. Temperature controlled sex-determination in turtles: ecological and behavioral aspects. Herpetologica 38:156–164.

Vogt, R. C., and O. Flores-Villela. 1992. Effects of incu-

bation temperature on sex determination in a community of Neotropical freshwater turtles in southern Mexico. Herpetologica 48:265–270.

Wade, M. J. 1998. The evolutionary genetics of maternal effects. Pages 5–21 *in* T. A. Mousseau and C. W. Fox, eds. Maternal effects as adaptations. Oxford University Press, Oxford.

Weisrock, D. W., and F. J. Janzen. 1999. Thermal and fitness-related consequences of nest location in painted turtles (*Chrysemys picta*). Functional Ecology 13: 94–101.

Wilbur, H. M. 1975. The evolutionary and mathematical demography of the turtle *Chrysemys picta*. Ecology 56: 64–77.

Wolf, J. B. 2000. Gene interactions from maternal effects. Evolution 54:1882–1898.

Zweifel, R. G. 1989. Long-term ecological studies on a population of painted turtles, *Chrysemys picta*, on Long Island, New York. American Museum Novitates 2952: 1–55.

Zwiers, F. W. 2002. The 20-year forecast. Nature 416: 690–691.

Associate Editor: Donald B. Miles